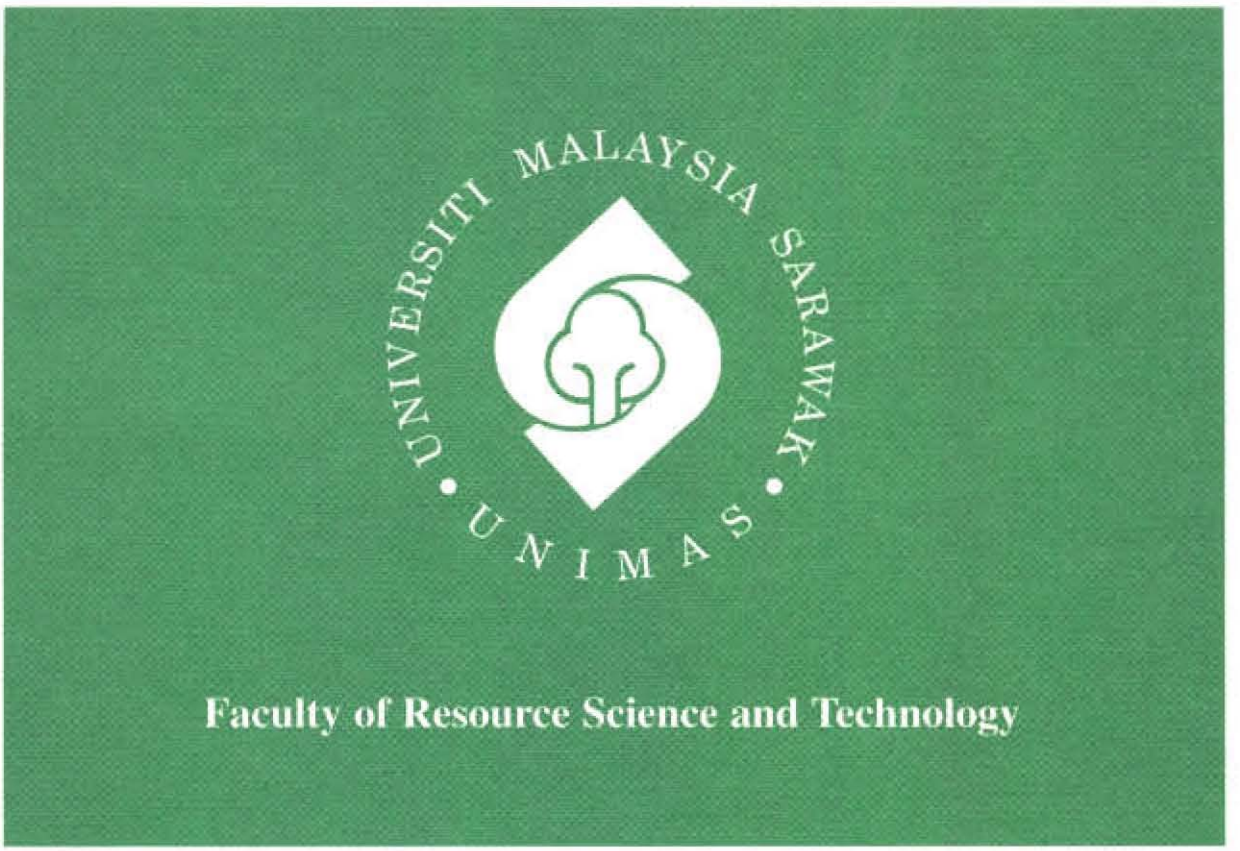

INDUCTION OF CALLUS AND SOMATIC EMBRYOGENESIS IN OIL PALM

(Elaeis guineensis Jacq.)

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Bachelor of Science with Honours
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Induction of Callus and Somatic Embryogenesis in Oil Palm (*Elaeis guineensis* Jacq.)

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ABSTRACT

Oil palm is the golden crop of Malaysia. Currently the oil palm industry is expanding. Large amount of true-to-type and uniform clonal material with superior trait are needed in order to establish oil palm plantation. Being a single stemmed monocot without vegetative propagation material for conventional method of propagation, the only solution is to develop tissue-cultured clonal plantlets. The present study was carried out to establish an *in vitro* culture of *Elaeis guineensis* Jacq. for the use in large scale propagation. An effective surface sterilization regime each for young fronds and seeds has been established. Young frond surface sterilized in 20% Clorox for 10 minutes and seeds 30% Clorox at pH 5.8 for 10 minutes resulted 100% axenic explants. For callus induction of callus on young frond explants, ½ MS media incorporated with 2,4-D (25, 50, 75 and 100 mg/L) alone and 3mg/L 2ip was used. NAA (25, 50, 75 and 100 mg/L) alone and incorporated with 3 mg/L 2ip was also used for frond callus induction. AC or PVP was added into the media to prevent browning. Callus was successfully induced from young frond explants cultured on ½ MS supplemented with 25 mg/L of NAA. Zygotic embryos was cultured in Y3 media with 1 and 2 mg/L of 2,4-D for callus induction. Callus derived from zygotic embryo explants were then used in a liquid suspension for somatic embryogenesis.

Key words: *Elaeis guineensis* Jacq., *in vitro* culture, axenic culture, callus induction, somatic embryogenesis.

ABSTRAK

Kelapa sawit merupakan tanaman utama Malaysia. Pada masa kini, industri kelapa sawit sedang berkembang maju. Kelapa sawit yang seiras dengan induknya dan klonal material yang unifom dengan ciri-ciri yang unggul amat diperlukan untuk ladang kelapa sawit. Sebagai pokok monokot yang berstem tunggal dan tanpa material propagasi vegetatif, satu-satunya penyelesaian terhadap masalah ini ialah menghasilkan benih tanaman klonal menggunakan teknik tisu kultur. Jadi, kajian ini dilaksanakan untuk menghasilkan rejim kalus induksi dan somatik embriogenesis bagi Elaeis guineensis Jacq. untuk kegunaan propagasi secara besar-besaran. Rejim yang efektif untuk mengsterilkan permukaan setiap pelapah muda dan biji benih telah diwujudkan. Pelepah muda yang disterilkan dengan 20% Clorox selama 10 minit dan biji benih yang disterilkan dengan 30% Clorox pada pH 5.8 selama 10 minit memberi hasil 100% eksplan yang axenik. Bagi induksi kalus eksplan pelapah dan biji benih, ½ MS media yang mengandungi 2,4-D (25,50,75 dan 100 mg/L) sahaja dan mengandungi 3 mg/L 2ip digunakan. NAA (25,50,75 dan 100 mg/L) sahaja dan dengan 3 mg/L 2ip juga turut digunakan. Kedua-dua cara ini digunakan untuk menginduksikan kalus daripada pelapah muda. Serbuk arang atau PVP ditambahkan dalam media untuk mengatasi pengunningan kalus. Kalus telah berjaya diinduksikan dari eksplan pelapah muda yang dikulturkan dalam media ½ MS ditambah dengan 25 mg/L NAA. Kalus yang diperolehi daripada eksplan zygotik embrio digunakan dalam suspensi cecair untuk somatik embriogenesis.

Kata kunci: Elaeis guineensis Jacq., in vitro kultur, kultur axenik, induksi kalus, somatik embryogenesis.

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LIST OF ABBREVIATIONS

2,4-D	2,4-dichlorophenoxy acetic acid
2ip	2-isopentenyladenine
BAP	6-benzyl amino purine
MS	Murashige and Skoog medium
NAA	Naphthalene acetic acid
PPM™	Plant Preservative Mixture
Y3	Eeuwens, 1978 medium
PVP	Polyvinylpyrrolidone
AC	Activated Charcoal

1 INTRODUCTION

The oil palm, *Elaeis guineensis* Jacq., is native to Africa. Its commercial value lies mainly in the oil that can be obtained from the mesocarp of the fruit - palm oil and the kernel of the nut - palm kernel oil. Palm oil is used mainly for cooking (cooking oil, margarine, shortening, etc.) and it also has non-food applications (soap, detergent, biofuels, etc.).

The Malaysian oil palm industry has expanded rapidly in the last forty years. Oil palm planted areas grew from 54,000 ha in 1960 to 3.08 million hectares in 1998. Palm oil production increased correspondingly from 94,000t to 8.3 million tones for the same period. This rapid growth was partly stimulated by progress in research and development, such as the discovery and subsequent adoption of the *tenera* variety to replace the *dura* as the commercial planting material (Anon, 2000a).

In order to establish plantation of genetically homogenous oil palm with superior trait, large amount of true-to-type and uniform clonal material are needed. Besides, oil palm being a single stemmed monocot without vegetative propagation material such as suckers no other conventional methods of vegetative propagation are possible for oil palm, the only solution is to develop tissue-cultured clonal plantlets.

The development of *in vitro* culture techniques, particularly the possibility of cloning monocotyledonous species by somatic embryogenesis has attracted many oil palm breeders. As early as 1970s, several teams were working on the condition for micropropagation of oil palm (Smith and Jones, 1970; Rabechault *et al.*, 1970). The regeneration of plants was reported in the following years (Jones, 1974; Rabechault and Martin, 1976 cited by Duval, 1997). Producing oil palm clones had several theoretical advantages. Cloning opened the way for large-scale plantation of oil palm with selected highest producing Dura x Pisifera progenies obtained in breeding programmes. Besides, it promises of having more homogeneous plantings and a possibility of having clones with architectural characters or disease tolerance clones (Noiret *et al.*, 1985 cited by Duval, 1997).

The first batch of tissue culture derived oil palm trees were planted by the Unilever group in Malaysia in 1977 (Corley *et al.*, 1982). Subsequently, the French group at IRHO-CIRAD field planted cloned palms in La Mc Station in the Ivory Coast in June 1978 (Lioret, 1982). It is thus more than 25 years since the first report on planting of tissue-cultured derived oil palm.

The appearance of fruit and floral abnormalities in clonal oil palm was first reported in 1986 (Corley *et al.*, 1986). Four clones supplied by a commercial company and planted in 1984 had high incidences of mantling and andromorphic male inflorescences. The same clones were also supplied to other research organizations and the abnormalities occurred with them (Anon, 2000b). Abnormalities in clonal propagated

oil palm might be due to high concentration of 2,4-D rountinely used for its culture as 2,4-D is reported to cause mitotic spindle abnormality (Bayliss,1973 cited by Sogeke, 1998). There is a renewal of interest in oil palm research; Corley *et al.* (1986) and Hartley, (1988) suggested the media used should be adjusted to overcome the abnormalities. Apart from the problem of fruit and floral abnormalities, the efficiency of oil palm tissue culture is low.

The objectives of this study are to i) develop an effective surface sterilization regime and culture medium to establish axenic (aseptic or contamination free) explants from field grown material of oil palm, (ii) induce callus formation in leaf and zygotic embryo explants and (iii) to induce somatic embryogenesis from callus, if time allowed.

2 LITERATURE REVIEW

2.1 Arecaceae

The oil palms (*Elaeis*) comprise two species of the Arecaceae, or palm family. They are used in commercial agriculture in the production of palm oil. The African Oil Palm *Elaeis guineensis* is native to west Africa, occurring between Angola and Gambia, while the American Oil Palm *Elaeis oleifera* is native to tropical Central America and South America (Anon, 2006b).

2.2 Oil palm – *Elaeis guineensis* Jacq.

The oil palm *Elaeis guineensis* Jacq. is widely grown commercially in South East Asia, Equatorial America, Africa and South Pacific (Latiff, 2000). In 1848, four seedlings were planted in Botanical Garden, Bogor in Java, Indonesia. Until the 1950s all the oil palms in Indonesia and Peninsular Malaysia were derived from these four trees and their offsprings. They are described as the Deli *dura*.

Due to the oil palm being the plant of commerce in Malaysia, it is only appropriate that it has to be improved continuously in its traits of interest. For example, breeding programmes are guided by the following objectives: increased oil yield per hectare, better oil quality, reduced height increment rate and pest and disease tolerance. Secondary traits of interest in selection include high early yield, kernel yield, yield profile,

carotene level, Vitamin E, physiological traits and Genotype x Environment (GxE) interaction (Kushairi and Rajanaidu, 2000 cited by Madon *et al.*, 2005).

2.2.1 Distribution and Ecology

Increasing level of greenhouse gases such as carbon dioxide, methane and chlorofluorocarbons (CFCs) have depleted the ozone layer and is a major cause of rising global temperature, unexpected flood and other climatic catastrophes. Admist such global threat Malaysia's sprawling oil palm plantation agriculture continues to play a protective role of forest cover. In 1998, over 500 million living and breathing oil palm trees provide a green belt spanning 3.08 millions hectares or about 11.0 % of the total land area in Malaysia.

Oil palms are no different from other forms of plant life in sequestering carbon dioxide from and returning oxygen to the atmosphere. The cumulative beneficial effects of the photosynthesis are significant considering the relatively high leaf area index of the perennial green cover provided by the oil palm plantations. Studies have shown that oil palm plantation are as effective as rainforest in acting as carbon sinks areas of dry matter that serve to absorb the harmful greenhouse gases from the atmosphere (Henson, 1999).

Oil palm plantation are capable of assimilating up to 36.5 tonnes of dry matter/ha/year, which is significant and more than the 25.7 tonnes of dry matter/ha/year

assimilated by rainforest. Being a C4 plant that produces oxaloacetic acid (C4-acid) in the first step of photosynthesis, oil palm trees generate a net sequence of carbon dioxide as opposed to forest, which only generate dynamic carbon dioxide equilibrium. A net sequestered of carbon dioxide means oil palm trees absorb more carbon dioxide from the atmosphere compared to the volume of carbon dioxide they emit to the air (Henson, 1999).

In Malaysia, oil palm can be grown on a wide range of soils. It is found that adequate soil moisture is more important than nutrient supply, which can be supplied artificially. In Peninsular Malaysia, the best areas are the coastal alluvial clay, and in Sabah, the riverine and coastal alluviums, and soils of volcanic origin (Anon, 2000a).

2.2.2 General Morphology

Mature trees of oil palm are single-stemmed, and grow to 20 m tall. The leaves are pinnate, and reach between 3-5 m long. A young tree produces about 30 leaves a year. Established trees over 10 years produce about 20 leaves a year. The flowers are produced in dense clusters; each individual flower is small, with three sepals and three petals. The fruit (plate 1) takes five to six months to mature from pollination to maturity; it comprises an oily, fleshy outer layer (the pericarp), with a single seed (kernel), also rich in oil. Unlike the Coconut Palm, the oil palm does not produce offshoots; propagation is by sowing the seeds.

The taxomomy classification of oil palm is as below:-

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Liliopsida
Order:	Arecales
Family:	Arecacae
Genus:	*Elaeis*
Species:	*Elaeis guineensis*
	Elaeis oleifera

Plate 1 Oil palm fruits
Bar = 1cm

2.2.3 Uses and economic importance

Global oil prices went through the roof and with the increasing realisation that emissions from fossil fuel were choking the atmosphere, the world started looking seriously for an alternative. The answer is biofuel, which includes biodiesel, and with that, the palm oil sector found its Holy Grail and planters immediately saw even better times ahead. Even without this prospect, palm oil is a RM30 billion-a-year revenue-earner. As the world's leading producer of palm oil, the most important component of biodiesel, Malaysia is a major new source of biofuel (Anon, 2006a).

As the major oil palm producer, Malaysia aspires to meet the requirement of consumer and food manufacturer for versatile and affordable edible oil. Continuous research and development has allowed one hectare of oil palms to produce an average of 4-5 tonnes of oil per annum with best fields giving as high as 7-8 tonnes. In comparison, other oil bearing crops produce only a quarter of that. Progressive efforts toward production efficiency coupled with natural lifespan of oil palm trees of 25 years ensure a sustainable and consistent supply of this essential food for mankind. In 2004, Malaysia produced 14.0 million tonnes of palm which accounts for about 47 % of global production. Almost 90 % of Malaysia palm oil is exported (Anon, 2004).

2.3 Plant Tissue Culture

The demonstration of hormonal control of differentiation (Skoog and Miller, 1957) and totipotency of single plant cell (Steward *et al.*, 1958) laid the foundation for clonal propagation and micropropogation of plant through tissue culture techniques (Kharkwal and Roy, 2004 cited by Madon *et al.,* 2005). The development of techniques and protocols to produce plant embryos asexually has had a huge technological and economical impact on agriculture systems, and presently these biotechnologies represent an integral part in the breeding programmes of agronomically important crops (Maraschin *et al.*, 2005 cited by Madon *et al.,* 2005).

Oil palm trees are heterogeneous so clonal propagation of elite palms with good traits such as high yield in fresh fruits bunch (FFB), high monounsaturated oil, short trunk or bigger fruit types is desirable. Plant yielded 30% higher than the average could be cloned (Hardon *et al.*, 1987 cited by Madon *et al.,* 2005). Vegetative propagation of palms is not possible, while propagation by tissue culture has led to formidable problems such as floral abnormalities, which can cause abortion of the fruits.

Oil palm cloning protocols were established in the 1970s (Jones, 1974; Rabechault and Martin,1976; Paranjothy and Rohani, 1982 cited by Madon *et al.,* 2005). Explants used in the early years were zygotic embryos (Jones, 1974) and later leaf tissue from 'cabbage' (very young leaves taken from above the apical meristem) of mature elite palms were utilized. This is due to its abundance and sanitary conditions (Rohani *et al.*,

2003 cited by Madon *et al.,* 2005). A better understanding of tissue culture procedures and process via related discipline such as plant morphogenesis, plant growth and development and cytogenetics etc. is needed to improve the efficiency of the process.

Vegetative propagation of the oil palm by tissue culture has three significant advantages over conventional breeding. Firstly, rapid multiplication of uniform planting material with the desired attributes, thereby providing the maximum return for investment and the requisite materials for meaningful agronomic experiments. Secondly, it offers a new opportunity in oil palm breeding by obtaining haploid palms from pollen/anther culture. Thirdly, it opens new avenues of research for oil palm biotechnology.

In Malaysia, the first batch of tissue culture derived oil palm trees were planted by the Unilever group in 1977 (Corley *et al.*, 1982). It is more than 28 years since the first successes oil palm tissue culture were announced. The commercial potential of the process was obvious from the start, and increase in yields of 30% was predicted (Corley *et al.*, 1986). In Malaysia, the early 1980s saw the establishment of several tissue culture laboratories set up by the plantation agencies to exploit the process for large-scale production of planting materials. Much of this effort was dampened in 1986 when the occurrence of abnormalities in some clonal palms became publicly known (Corley *et al.*, 1986).

The initiation of callus from leaf or root tissue is generally on a medium containing a high concentration of 2,4-Dichlorophenoxy acetic acid (2.4-D) (Duval *et al., 1988*; Blake, 1983; Rabechault and Martin, 1976; Smith and Thomas, 1973 cited by Sogeke, 1998). At high concentration, 2,4-D and 2,4,5- Trichlorophenoxy acetic acid (2,4,5-T) are phytotoxic to broad leaf plants and used as herbicides (George and Sherrington, 1984 cited by Sogeke, 1998). Furthermore, cytological examination of callus from roots of oil palm grown on 2,4-D medium contained a high proportion of polyploidy and aneuploid cells (Smith and Thomas, 1973 cited by Sogeke, 1998). Researcher have successfully induced somatic embryogenesis from callus which was developed from medium containing 2,4-D (Jones, 1974; Rabechault and Martin, 1976; Paranjothy and Othman, 1982; Nwankwo and Krikorian,1983; Blake, 1983; Duval *et al.,* 1988 cited by Sogeke, 1998).

The presence of abnormalities, or somaclonal variation in tissue-culture derived oil palm, however, gave rise to a new outlook in research on oil palm tissue culture. Industries were investigating the cause of these abnormalities, especially the ones affecting flower development as this resulted in poor quality fruits being produced and thereby, reducing the oil yield. It was a difficult task to overcome abnormalities as the phenomenon of somaclonal variation, although widespread in all plants which have been cultured *in vitro* (Scowcroft, 1985 cited by Cheah, 2003), is not well understood. Researcher has looked into the molecular approaches for example attempt at applying recombinant DNA enabled techniques in the search for a marker for the mantled flower abnormalities, molecular probes employing restriction fragment length polymorphism

(RFLP) and employing of random amplified polymorphism DNA (RAPD) (Cheah and Wooi, 1995 cited by Cheah, 2003).

In order to provide greater focus in the efforts made to dissect the genetic basic of somaclonal variation, work on flower development was initiated as the abnormalities which occur during this stage were great concern to the industry. The technique of mRNA fingerprinting confirmed that differential gene expression occurred during flower development in oil palm (Rajinder and Cheah, 2000 cited by Cheah, 2003)

Now, about 20 oil palm laboratories are in operation throughout the world with capacity ranging from 10,000 – 200,000 plantlets per year (Zamzuri *et al.*, 1999 cited by Cheah, 2003). As compared to seed production, tissue culture of oil palm offers several advantages (Sogeke, 1998). It allows rapid multiplication of uniform planting materials with desired characteristics. This enables improvement of planting materials using existing individuals which have all or most of the desired qualities such as good oil yield and composition, slow vertical growth and disease resistance. Additionally, it also opens new avenues for producing novel planting materials via genetic engineering, because tissue culture is the means for regeneration of tissues transformed with genes for traits of interest.

Rohani *et al.*, (2000) reported that recent clonal performance has been very encouraging and a number of investigators have reported clones to be more uniform than DxP seedling, indicating that the clones are true-to-type. It has been demonstrated that